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(54) Title: METAL COMPLEXES OF ALOE EXTRACTS FOR SKIN AND HAIR

(57) Abstract

Methods are provided for preparing compositions suitable for protecting irritated or damaged skin from further oxidative and biochemical damage and thus permitting natural healing processes to progress, for accelerating the rate of healing of burns and surgical wounds, and for increasing the size of hair follicles and the rate of hair growth. The compositions generally comprise complexes formed by the complexation of aloe vera with ionic metals such as copper(II) salts and tin(II) salts.

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METAL COMPLEXES OF ALOE EXTRACTS FOR SKIN AND HAIR

Background of the Invention

Extracts from the plant aloe have a traditional use for the treatment of skin and skin injuries. Liquid obtained from the epidermis of leaves of Aloe forms a gel that can be applied to skin injuries and wounds. For ease of use, the aloe extract may be processed to a dry powder which can be subsequently restored to a gel form by the addition of water.

Controlled laboratory studies of the healing actions of the preferred aloe extract, commonly named aloe vera, however, provide a confusing picture of its preferred ability to stimulate the skin healing process. In these studies aloe vera gel has variously been reported to stimulate the skin healing process, to have no effect, or even to delay wound healing.

For example, Rodriguez-Bigas et al., Plast. Reconstr. Surg. 81: 386-389 (1988), found aloe vera gel to accelerate the healing of full-thickness burns in guinea pigs. Similarly, Davis and Maro, J. Am. Podiatr. Med. Assoc. 79: 24-26 (1989), reported aloe vera to improve wound healing in diabetic mice. On the other hand, Schmidt and Greenspoon, Obstet. Gynecol. 78: 115-117 (1991) reported aloe vera gel to delay the healing of women after cesarean delivery or laparotomy for gynecologic surgery from an average of 53 days using standard wound management techniques to an average of 83 days in women treated with aloe vera gel. Watcher and Wheeland, J. Dermatol. Surg. Oncol. 15: 188-1195 (1989) found no beneficial effect of aloe vera gel on the healing of surgical wounds in pigs. Kaufman et al., J. Burn Care Rehabil. 9: 156-159 (1988), concluded that aloe vera gel hindered the healing process of second degree burn wound in guinea pigs. Bracken et al., J. Occup. Med. 27: 733-739 (1985), found that alo vera gel did not improv the healing of hydrofluoric acid burns in rats.

Currently, the general consensus seems to be that aloe vera extracts possess, at best, only a very mild healing action and are ineffective for the healing of more serious wound and injuries.

The low efficacy and inconsistency of results with aloe vera gels has led to efforts to enhance a healing effect by various methods. Fulton, J. Dermatol. Surg. Oncol. 16: 460-467 (1990), reported improved skin healing by using a stabilized aloe vera gel impregnated into a polyethylene oxide dressing. Davis et al., J. Am. Podiatr. Med. Assoc. 79: 559-562 (1989), incorporated aloe vera gel into Eucerin cream and found improved healing of surgical wounds in rats.

Restoring the function of damaged and wounded skin continues to be a major health problem despite the development of various medications. Many approaches to skin-healing that are currently being developed, such as the production of growth factor proteins and pharmaceutical drugs with wound healing properties, and occlusive dressings for wounds, are beyond the economic reach of many patients. A major need exists for improved and low cost skin-care products and medicaments in less-developed countries, particularly in the tropics, where conditions such as inadequate health care, widespread skin fungal diseases, and the use of flammables such as kerosene for cooking and similar conditions give rise to high incidents of serious skin injury. For such countries, there is a critical need is to produce effective medicines that can be produced at very low cost using readily available materials. Even in more developed countries the increasing demands for cost-containment in medical services necessitate the development of low-cost products for skin care and pharmaceuticals for wound healing. Procedures such as hospitalization for the treatment of diabetic skin ulcers are increasingly being restricted. Thus, improved and cost-effective treatments for wound healing are required for the future.

Delayed healing or incomplete healing in humans and ther animals caus s additional pain and suffering for th patient and markedly increases wound c mplications and medical

costs. Often the wound continues as a chronic sore that requires extensive attention and medical care to control infection and tissue necrosis. Even when such wounds finally heal, the wound area is frequently devoid of the ability to respond to tactile stimulation and is often filled with excessive deposits of immature collagen that produces permanent scarring. The urgent need for improved wound-healing compositions extends to wounds generated by surgical procedures. The success of surgical procedures, especially in very ill or elderly patients, is typically a function of the adequacy and speed of post-surgical healing.

Another aspect that can impair the normal healing response is excessive inflammation of injured or wounded skin. While the inflammatory process and its concomitant influx of white cells into the afflicted area are an integral part of the natural healing process, in some cases the inflammatory process becomes excessive and delays healing. The wounded tissue becomes locked in an early phase of the healing process and cannot proceed to completion. In such instances, compounds with anti-inflammatory activities are used to allow the process to proceed normally. One promising approach for the therapeutic treatment of conditions associated with inflammation and impaired wound healing has been the use of metal ions complexed to organic molecules or amino acids, amino acid derivatives and peptides. Some of these complexes possess anti-inflammatory activity, while others possess both anti-inflammatory activity and healing actions. Yet other complexes reportedly possess hair-growth stimulating actions in addition to anti-inflammatory and/or healing activities, as described in, for example, applicant's co-owned pending patent application serial no. 07/954,620, which is incorporated in its entirety by reference herein.

The use of copper salts or complexes as anti-inflammatory agents for the healing of stomach ulcers in the treatment of patients suffering from acute or chronic arthritis dates back to the 1940's and 1950's (see, e.g., reviews by Sorenson, Inflammation, 3:317-331 (1976); Agents and Actions 8:305-331 (1981), and Comprehensive Therapy

11:49-64 (1985)). The use of copper salts and complexes, such as c pper-salicylate complex, seems to have been aband ned, apparently due to the early promise of the steroidal anti-inflammatories, such as hydrocortisone. Other complexes of copper with amino acids (tryptophan, lysine), with non-steroidal anti-inflammatory drugs (indomethacin, ketoprofen, acetylsalicylic acid) or with fatty acids (oleic, lauric and caprylic acids) have been studied but, despite their promise, were rarely developed beyond the preclinical phases, apparently due to problems of irritation, toxicity, and inadequate efficacy.

While many copper-complexes have been reported to possess anti-inflammatory properties, a more limited group have been reported to also possess healing actions. Heintze (U.S. Pat. No. 4,123,511) reported that a copper oleate complex had anti-inflammatory and skin healing activity. Sorenson (U.S. Pat. No. 4,440,754) describes the use of complexes of copper(II) salts and amino acids, such as tryptophan or lysine, or with organic molecules such as 3,5-diisopropylsalicylic acid, acetylsalicylic acid or salicylic acid, to prevent and heal gastrointestinal ulcers. Using a wound-healing model, Townsend and Sorenson (Sorenson et al., Agents and Actions 8:305-325 (1981)) found salicylate-copper to accelerate the rate of healing and to improve the quality of healing of surgically-induced ulcers in rats. Also, Sorenson wrote (ibid. and Inflammation 3: 317-331 (1976)) that Townsend demonstrated that copper(II)-(tryptophan)2 increased the rate of ulcer healing in a surgically-induced ulcer model. The increased healing was purportedly due to a more rapid re-epithelialization of the wound and an increase in the quantity and quality of the collagen. Fine collagen fibers in a normal orientation developed in treated animals, in contrast to non-treated animals in which the new collagen was very dense and composed of thick, wavy disoriented bundles, resembling scar tissue.

Federici and Bertolotto (EP 450,398 and IT 9,019,948) reports that chondroitin sulfate-copp r(II) complexes possess d anti-inflammat ry activity. European

Patent N . EP 66,283 discloses "eustatic" comp sitions which contain a non-toxic metal i n (including copper) and a glycosamino-glycan of hyaluronic acid or chondroitin sulfate useful as a cicatrizant (wound healing by closure).

UK Patent Application GB 2 044 265 describes metal complexes (including copper) of adenosine triphosphate as aiding the recovery of bone tissue in cases of fractures as well as in osteoporosis and bone cysts.

Konishi (US Pat. No. 4,461,724) reports that the tetrapeptide Gly-Ser-His-Lys and peptides of related structures possess anti-inflammatory and healing actions when complexed with metals such as ionic copper and zinc.

Yu (U.S. Patent 4,053,630) discloses the use of cysteic acid and its derivatives cysteine sulfinic acid or homocysteic acid, chelated to metal ions such as ferric, cupric, zinc or aluminum, to form compositions that alleviate symptoms of diseases characterized by defects of keratinization and achieved a remission of ichthyosis, dandruff and acne. Bertelli (U.S. Patent 4,156,737) suggests that copper complexes of p-aminomethyl-benzene-sulfonamide possess healing and protective effects on skin burns. Van Scott (U.S. Patent 4,283,386) reports that metallic (copper, zinc, or aluminum) salt forms of cysteic acid, cysteine sulfinic acid and homocysteic acid have therapeutic actions that produce remissions of dry and broken skin, keratoses, warts and palmar and plantar hyperkeratosis.

Niwa (Dermatologica 179 S1: 101-106 (1989)) and Bergren et al. (Am. Surg., 54: 333-336 (1988)) found that the anti-inflammatory protein Cu, Zn-superoxide dismutase also acts to enhance healing processes.

Pickart (see, e.g., PCT Publications WO 91/14437, WO 91/12267, WO 91/05797, WO 91/03488, WO 89/12441, WO 88/26448, WO 88/08851, EP Patents EP 190,736, EP 189,182; and U.S. Pat. No. 4,767,753) describes the synthesis and use of metal complexes of Gly-L-His-L-Lys as anti-inflammatory and healing agents.

A numb r of metal complexes have b en us d to promote hair growth. Yamashiki (Japan Pat. 70018997) used a

complex of copper-pantothenate to purportedly promote growth of hair roots and promote skin functions. Morelle (U.K. Pat. GB 2097256, DE Pat. 32212448) used amino acid derivatives (N-butyryl amino acids) complexed with copper and other metals for cosmetic and therapeutic purposes, including use as hair and skin stimulants. Banfi et al. (U.S. Pat. No. 4,503,047) disclose a composition containing primarily one or more sulfur-containing amino acid(s) and copper(II) ions plus smaller amounts of allyl isothiocyanate and rhodanide ions to produce hair-growth stimulating actions. Pickart (e.g., WO 91/07431, 88/08695 and EP 288,278) found a number of metal complexes of derivatives of Gly-L-His-L-Lys to increase hair follicle size and the rate of hair-growth.

Despite the therapeutic promise of the above-mentioned metal complexes, toxicity and tissue irritation occur with many metal complexes (see, e.g., Johnson et al., Inorg. Chem. Acta, 67: 159-165 (1982); Pickart et al., Biochem. Pharm., 32: 3868-3871 (1983); and Pickart et al., Lymphokines 8: 425-446 (1983)). For example, while copper-salicylate complexes and numerous copper-salicylate analogs possess anti-inflammatory activities, other salicylate analogs such as the copper(II) complex of salicylaldehyde benzoyl hydrazone are highly toxic to tissues. Similarly, copper(II)-Gly-L-His-L-Lys supports cellular viability and possesses anti-inflammatory and healing actions, yet close synthetic aroylhydrazone analogs of its copper-binding region are extremely toxic to cells and tissues.

Another problem with copper complexes for therapeutic use concerns the binding affinity of copper ion to the complexing molecule. While a defined copper-complex can be synthesized, its therapeutic use places the complex in the physiological milieu of the tissues where a plethora of literally hundreds of compounds compete for binding to the copper ion, which can form electrostatic bonds to as many as six separate molecules. If the copper is removed from the complex and becomes loosely bound, then tissue irritation occurs (see Raju et al., J. Natl. Cancer Inst., 69: 1183-1188 (1982)).

Further complications arise when such metal complexes are formulated into carrier creams or ointments. Various chemicals are added to the formulations to increase adherence to skin and wound surfaces and to enhance the penetration of the complexes into the target tissue. Yet, since many of these substances also bind to the metals, the expected therapeutic benefits may be nullified or significantly attenuated. Also, detergents such as sodium dodecyl sulfate are used to help blend oil and water phases of the emulsions and stabilize the formulations. However, such detergents are themselves tissue irritants that can delay healing.

Another problem encountered with many of the metal complexes intended for therapeutic use is that they cannot be heat-sterilized; hence, to meet safety requirements, high concentrations of antimicrobial chemicals must be added during manufacture to inhibit the growth of microorganisms and the transmission of viruses. These antimicrobial agents may also inhibit the viability and function of a host's cells such as macrophages and fibroblasts that are involved in the maintenance and repair of skin and other tissue, and thus these agents may retard the healing response.

What is needed in the art are compositions useful in tissue protection, tissue healing, and/or stimulating hair growth, which compositions could be conveniently produced and at low cost. Preferably, the compositions could be sterilized without loss of bioactivity and could be formulated for topical application without the use of detergents or other potentially irritating compounds. The ideal composition would also adhere well to skin and other materials such as wound dressings (for example, adhesive bandages). To speed the time and expense required for regulatory approvals, the compositions would be prepared from materials that are generally recognized as safe by regulatory agencies and thus could be used with minimal safety concerns and regulatory barriers. Quite surprisingly, the current invention fulfills these and other related needs.

Summary of the Invention

The present invention provides compositions and methods for accelerating the healing of topical wounds and skin irritation, for protecting skin from damaging effects of oxidation, and for increasing the size of hair follicles and the rate of hair-growth in warm-blooded animals. The compositions useful in these methods, including pharmaceutical compositions, are prepared from an aloe gel, such as the gel extract of aloe vera, that is complexed with an ionic metal.

Thus, in one aspect the invention provides methods for preparing the pharmaceutical compositions useful in accelerating the healing of topical wounds or increasing hair follicle size and hair growth in a warm-blooded animal. An aloe gel is combined with an aqueous solution of a metal salt, then adjusted to an acceptable acidity range for skin products. Typically, the aloe gel is obtained as a dry powder from commercial sources then reconstituted into a gel with water. The aloe gel is then complexed to an ionic metal such as copper(II), tin(II), zinc(II), etc. This procedure causes a very substantial and significant increase in the wound healing properties of an aloe gel and also produces compositions that markedly stimulate hair growth.

The resulting aqueous mixture is composed of complexes of the aloe gel and the metal ions. The amount of water in the mixture is adjusted to produce a thick but spreadable gel. The resultant gel may be used directly or combined with a pharmaceutically acceptable carrier to form a cream or lotion, in a concentration of from about 10% to about 50% aloe gel-ionic metal complex or more. The preparation may be pasteurized, if desired, without destroying the healing or hair growth stimulating activity of the aloe gel-metal complex.

In other embodiments the invention provides methods for enhancing the recovery of skin of a warm-blooded animal from wounds, such as surgical incisions, burns, inflammation or minor irritation due to oxidative damage, etc. The methods comprise administering to the skin wound or irritation a

therapeutically or, in some cases a prophylactically effective amount of a composition which comprises the aloe gel-ionic metal complex. Due to the gel-like composition of the invention, the material is particularly easy to apply to irregular surgical wounds and incisions.

Yet other embodiments relate to compositions and method for increasing hair follicle size and the rate of hair growth in warm-blooded animals, such as humans. The methods comprise administering to the skin in the area in which hair growth is desired an amount of aloe gel-ionic metal complex sufficient to increase hair follicle size and the rate of hair growth in said animal. The composition is administered in a variety of ways, such as topically in a gel or cream, by local injection, etc., and will be typically applied on a regular basis, e.g., daily, until hair growth is observed and for a time thereafter sufficient to maintain the desired amount of hair growth.

Description of the Specific Embodiments

Compositions and methods are provided by the present invention for topical skin treatments to protect damaged skin and thereby allow natural healing processes to proceed, to enhance tissue regenerative processes in the skin of warm blooded animals, and to stimulate hair growth in warm blooded animals. The composition are formed by the complexation of aloe gel and ionic metals such as copper, zinc, tin or the like. Methods are provided for improving the recovery of damaged skin, accelerating the healing of burns or surgical incisions, and stimulating hair growth in warm-blooded animals.

The aloe gel-ionic metal complexes of the present invention are prepared from aloe gel, or extracts from the leaves of plants which are members of the Aloe family (e.g., *A. perryi*, *A. barbadensis*, *A. capensis* and particularly *Aloe vera*), or a dried aloe extract in a powdered form which can be

reconstituted. Aloe extracts, gels and powders are widely available from commercial sources.

To produce the aloe gel-ionic metal complexes useful in the present invention, the aloe gel, or the aloe powder which is converted to a gel by addition of water, is then complexed via admixture with one or more ionic metals, such as copper, zinc, indium or tin or the salts thereof, such as sulfate, acetate, phosphate, chloride, citrate, succinate, oxalate, cinnamate, tartrate, fumarate, maleate, glutarate, etc. By complexed is meant that the aloe gel and metal ions form electrostatic bonds, although this mechanism is offered by way of explanation only and not by way of limitation.

In one method for preparing the aloe gel-metal complex, the aloe gel is mixed with sufficient water (generally at about room temperature, about 22-25°C) to form a thick gel. To obtain this gel, dry aloe powder (1 gram) is mixed with about 2 ml water. The gel is then mixed with a aqueous solution of an ionic metal salt, e.g., copper(II) chloride or tin(II) chloride, at a salt concentration of about 10 to 50% (w/v), more preferably about 20% (w/v). The volume of metal salt solution added is generally that amount needed to obtain a final metal concentration in the paste of from about 0.3% up to about 1.2% (weight/weight) or more. As the addition of the metal salt may reduce the pH of the gel, the pH of the gel is then raised to about 6.5. This pH is useful for skin preparations but other pH levels near neutrality or in the slightly acidic range are also efficacious.

The resultant thick gel can be applied directly to the skin or is formulated into skin creams and lotions at concentrations of usually 10 to 50% (w/w) although higher concentration are also effective.

The aloe gel-ionic metal complexes of the invention may be administered for a variety of therapeutic, prophylactic or cosmetic uses to humans or in veterinary applications to other warm-blooded animals. Among veterinary animals particularly well suited for treatment with the present compositions ar sp ci s of equine, bovine, porcine, ovin , caprin , canine, avian, feline, etc.

The compositions and pharmaceutical preparations therefore are intended for local, topical, oral or parenteral (e.g., subcutaneous injection) administration for prophylactic and/or therapeutic or cosmetic treatment. Preferably, the pharmaceutical compositions are administered locally, e.g., topically, as a paste, cream or salve.

For administration to warm-blooded animals, the aloe gel-ionic metal compositions will typically be sterilized and incorporated in pharmaceutical or veterinary formulations. Compositions which comprise the aloe gel-metal complexes can be sterilized by conventional, well known sterilization techniques, e.g., pasteurization, without substantially adversely affecting the biological activity of the aloe gel-ionic metal complexes. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions and as necessary to prepare compositions for convenient administration, such an pH adjusting and buffering agents, and delivery vehicles. Actual methods for preparing pharmaceutically administrable compounds will be known or apparent to those skilled in the art, some of which are described in detail in, for example, Remington's Pharmaceutical Science, Mack Publishing Co., Easton, PA pp. 428-429 (1981), which is incorporated herein by reference.

Depending on the intended mode of administration and the intended use, the compositions may converted to solid, semi-solid, or liquid dosage forms, such, for example, as powders, granules, crystals, liquids, suspensions, liposomes, pastes, cremes, salves, etc., and may be in unit-dosage forms suitable for administration of relatively precise dosages. The compositions may include a conventional pharmaceutical carrier or excipient and, in addition, may include other medicinal agents, growth factors, wound sealants, carriers, etc., as further described below.

For semi-solid compositions, as would be appropriate for pastes and creams intended for topical administration, the aloe gel-ionic metal complexes can be provided separately or may be compounded with conventional nontoxic carriers such as, for example, squalane, glycerol sterate, polyethylene glycol,

cetyl alcohol, stearic acid, and propylene glycol, among others. Such comp sitions may contain about 5-100% active ingredient, more preferably about 10-50%. The concentration of the aloe vera gel-ionic metal complexes in these formulations can vary widely, and will be selected primarily by intended use, viscosities, etc., in accordance with the particular mode of administration selected and intended treatment. The composition or formulation to be administered will, in any event, contain a quantity of the aloe gel-metal complexes sufficient to achieve the desired therapeutic or prophylactic effect in the subject being treated.

The tissue healing compositions of the invention are administered to a warm-blooded animal, such as humans, already suffering from a wound, oxidative skin damage, inflammatory skin lesions, as described above, in an amount sufficient to allow the healing process to proceed more quickly than if the host were not treated. In the case of an animal suffering from decreased hair follicle size and impaired hair growth, the compositions of the invention are administered in an amount sufficient to increase hair follicle size and the rate of hair growth. Amounts adequate to accomplish these effects are defined as a "therapeutically effective doses." Amounts effective for these uses will depend on the severity of the wound, sore, etc., in the case of wound healing, and the extent of decreased follicle size in the case of impaired hair growth, and the general state of health of the patient being treated, but will generally range from about 1 mg to about 25 mg per day of aloe gel-metal complex per day per square centimeter of wound site, with dosages of from about 5 mg to about 10 mg per day per square centimeter of wound site being more commonly used. Maintenance dosages over a prolonged period of time may be adjusted as necessary. For veterinary uses higher levels may be administered as necessary. Determining actual amounts of the aloe gel-metal complexes necessary to treat a particular wound or condition as described ab ve will be through standard empirical m th ds well known in the art.

In prophylactic applications compositions containing the aloe gel-ionic metal complexes are administered to a host susceptible to or otherwise at risk of skin lesions or similar damage, to enhance the host's own wound healing or anti-oxidative capabilities. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the host's condition and general state of health, but generally range from about 0.1 mg to about 10 mg per day per square centimeter of skin, more commonly from about 1 mg to about 3 mg per cm^2 of skin per day. Single or multiple administrations of the compositions can be carried out.

The aloe gel-metal complexes of the invention may be administered in relatively large amounts without serious side effects, although indiscriminate use may produce discoloration of the skin. In instances where the compositions are administered to inhibit oxidative or biochemical damage to the skin or to those suffering from only mild irritation or inflammation of the skin, the dose may be adjusted accordingly to lower maintenance levels.

The compositions of the invention, including pharmaceutical compositions may be administered alone or as adjunct therapy or prophylaxis. The aloe vera gel-ionic metal compositions can be used in combination with other compositions, such as described in commonly owned copending US Patent 5,382,431 and patent applications USSN 08/218,392 and 08/219,681, each of which is incorporated herein by reference in its entirety, or with other growth factors known to improve other aspects of healing. In this manner, a synergistic effect may be attained that yields a clinical efficacy greater than that realized with any single factor. Further, while the compositions described herein stimulate a spectrum of healing processes, clinical wounds may differ considerably in their properties and healing patterns, leading one to utilize a combination of a composition described herein and another factor. For example, nerve regeneration is defective in many burns and thus n can add a specific nerv growth factor to supplement the composition t enhance nerve regrowth into the

burn area. Examples of fact rs with other reported healing pr perti s include epidermal gr wth factor, fibroblast growth factor, nerve growth factor, transforming growth factors, angiogenic growth factors, heparin, fibronectin, fibrin, platelet-derived growth factor, enzymatic superoxide dismutase, extracts of blood or factors from the blood, and other similar factors.

The following examples are offered by way of illustration, not by way of limitation.

EXAMPLE I

Preparation of Active Aloe Vera Gel-Ionic Metal Complexes

This Example describes methods used in the preparation of the aloe vera gel-ionic metal complexes having biological activities described further below. Aloe vera gel extract in a dry powdered form was purchased from Agro-Mar, Lakes, Nevada. Cupric chloride was from Sigma Chemical Company, St. Louis, MO while tin (II) chloride, 99% pure was purchased from Aldrich Chemical Company, Milwaukee, WI.

In one method for preparing the aloe vera gel-ionic metal gel complex, the aloe vera gel was mixed with sufficient water (at room temperature, about 23°C) to form a thick gel. To obtain this gel, dry aloe vera powder (1 gram) was mixed with 2 mls water. The gel was then mixed with a aqueous solution of a metal salt (copper(II) chloride or tin(II) chloride at a salt concentration of 20% (w/v). The volume of metal salt solution added is that amount needed to obtain a final metal concentration in the paste of 0.3 to 1.2% (weight/weight). The addition of the metal salt reduced the pH of the gel to about 2.9. The pH of the gel was then raised to 6.5 by careful addition of 1 N sodium hydroxide.

The aloe vera gel-copper complexes were used as skin protective agents that served as a skin barrier over damaged or irritated skin. Th gel adhered t the skin and formed a protective barrier, while the complexed copper serves to

impart an antioxidant activity to the mixture. As shown below, damaged or irritated skin h aled strikingly fast r after treatment with such a composition.

As also described further below, the aloe vera gel-copper and aloe vera gel-tin were used to promote hair growth and the enlargement of hair follicles. In hair growth models in mice, application of these complexes to the skin produced a marked stimulation of hair growth after 8 to 12 days.

Other types of aloe vera gel-metal complexes, e.g., aloe from other plants, other salts of the metals, such as sulfate, acetate, phosphate, and so forth would be expected to work similarly.

EXAMPLE II

Healing of Surgical Wounds with Aloe Vera Gel-Copper Complexes

This Examples describes the use of a gel prepared with the aloe vera gel-copper complexes to hasten the healing of surgical incision wounds in animals.

Surgical incisions (1.25 cm) were made on the backs of anesthetized, 35 gram, Swiss-Webster mice. Immediately after surgery and 24 hours later, the wounds were covered with a thin film of the paste containing the active aloe vera gel-copper complex in Example I above. Control wounds were untreated or were treated with aloe vera not containing the copper. As seen in Table 1, wounds treated with the active aloe vera gel-copper complex healed faster than control wounds. In contrast, aloe vera gel without copper ion had no significant healing action in this model. Since rapidly healing wounds tends to contract and become more rounded, the healing activity can be related to the length of the wound after 9 days. Each group consisted of eight mice.

Table 1: Effect of aloe vera gel-copper complex on incision length.

Test group	Length of wound after 9 days (cm.)
Control	0.87 ± 0.15
Aloe vera gel-no copper	0.90 ± 0.16
Aloe vera gel-1.0% copper complex	0.46 ± 0.03
Aloe vera gel-0.3% copper complex	0.54 ± 0.10

EXAMPLE III

Healing of Burn Wounds with Aloe Vera Gel-Copper Complex

This Example demonstrates the increased healing of burn wounds in animals using the aloe vera gel-copper compositions applied topically.

Second-degree burns were induced on the shaved backs on anesthetized mice by placing a circular (1.25 cm diameter, wound area = 1.22 cm^2) brass rod (temperature 100°C) in contact the skin for 7 seconds. Immediately after burning, and 24 and 48 hours later, the wounds were covered with a thin film of the paste containing the active aloe vera gel-copper complex of Example I above. Control wounds were untreated or were treated with an aloe vera gel without copper. Wounds were traced on anesthetized mice, digitized from an computerized scanning bed, and area calculated from the computerized pixel number. Burns treated with the active aloe vera gel copper complexes showed less post-burn inflammation and healed markedly faster than untreated control wounds or wounds treated with an aloe vera gel without copper.

Table 2: Effect of aloe vera gel-copper complex on burn wounds.

Test group	Area of wound after 15 days (cm^2)
Control	0.85 ± 0.07
Aloe vera gel without copper	0.79 ± 0.14
Aloe vera gel-0.3% copper complex	0.52 ± 0.08
Aloe vera gel-1.0% copper complex	0.46 ± 0.04

EXAMPLE IV

Reduction in Post-Burn Inflammation of Skin

This Example demonstrates the ability of aloe vera gel-copper complex to reduce inflammation associated with mild skin burns.

Very mild thermal burns were induced on the shaved backs of anesthetized mice (8 mice in each group) by a placing a circular (1.25 cm diameter, irritated area = 1.22 cm^2) brass rod (60°C) in contact the skin for 5 seconds. This produced a mild skin irritation characterized by redness and swelling, but rarely a loss of skin tissue. Immediately after inducing the thermal injury, the irritated area was covered with a thin film of the gel containing one of the following: aloe vera gel without copper, aloe vera gel with 0.3% copper ion, aloe vera gel with 1.0% copper ion. Control wounds were untreated. Wounds were observed at daily intervals. At day 3, the untreated thermal injuries were still reddish and swollen while the skin with any of the three aloe vera gel-copper complexes had minimal reddishness and swelling. Aloe vera gel without copper gave a slight improvement in skin condition but much less than th copp r-containing complex s.

EXAMPLE V

Pasteurization of Active Aloe Vera Gel-Copper Composition

Pasteurization consists of heating a solution to 160° for 30 minutes which kills all but the hardiest microorganisms. For this test, various aloe vera gels were complexed with copper chloride by the methods described above in Example I. The resultant gels were then heated to 160°F for 30 min. After cooling to room temperature, the gels were applied to surgical incision wounds in mice in the manner described in Example II. Healing activity was similar to that observed with unpasteurized active composition, as shown in Table 3. Each group contained six mice.

Table 3: Effect of pasteurization on active complex.

Test group	Length of wound after 9 days (cm)
Control	0.94 ± 0.10
Aloe vera gel no copper	0.91 + 0. 14
Aloe vera gel-1.0% copper complex	0.49 ± 0.11
Aloe vera gel-0.3% copper complex	0.53 ± 0.11

EXAMPLE VI

Adherence of Aloe vera gel-Copper paste to Skin

This Example describes the use of a aloe vera gel-copper active complex to improve the adherence of a wound dressings to the surface of the skin. The aloe vera gel-copper complexes can thus be used to more effectively cover and seal wounds with dressings. Also, many tapes used to hold medical sensors to th skin or catheters in veins cause skin irritation. The incorporation of an aloe vera gel-copper

complex into the adhesive used on such tapes could reduc such skin irritation and injury.

For testing, the wound coverage area of a Band Aid™ brand adhesive bandage was cut away from the adhesive area. The wound coverage area of the tape, which has no skin adhesive qualities, was covered with the aloe vera gel with 0.3% copper ion. The paste-covered tape was applied to the upper arm of humans and left in place during normal work functions in an office. The tape adhered well to the skin during the subsequent 8 hour test. This demonstrates that such wound-healing pastes possessed significant adhesive properties on human skin.

EXAMPLE VII

Stimulation of Hair Growth

This Example describes the use of compositions containing aloe vera gel-copper complexes to stimulate the growth of hair follicles in warm blooded animals. The model used in this test was a mouse model that has been found to successfully predict the therapeutic response in humans (see, e.g., U.S. Pat. No. 5,118,665, which is incorporated herein by reference). Hair growth in mammals proceeds through actively growing stages (anagen) followed by dormant stages (telogen). The test method generally involved applying the hair growth stimulant to the skin of mice in telogen phase. Female, Swiss-Webster mice begin a telogen phase at about 45 days of age that lasts until about 90 days of age. After application of the active substance, enhanced hair growth is noted within 10 to 14 days. For this test, mice 50 days of age were used.

Aloe vera gel compositions containing copper(II) and tin(II) were tested. For testing, the aloe vera gel-metal pastes were mixed with saline (25% aloe vera gel composition and 75% physiological saline by weight). Mice were shaved, then 0.05 ml of the mixture was infiltrated immediately below the skin by injection. Control mic were inj cted with an equal volume of saline. Each gr up contained 10 mice. After

12 days, the groups were compared. The percentage of mice with hair gr wth at the injection site and the relative strength of the hair growth response (on a scale of 1 to 5 where 1 is barely noticeable growth and 5 is very strong hair growth) were determined. The results, shown in Table 4, indicate that the compositions were active hair growth stimulants, with aloe vera gel-tin(II) complexes being the most effective agent.

Table 4: Stimulation of hair growth by Aloe vera gel-Metal Complexes.

	Percent with hair growth at injection site	Average intensity of hair growth
Control mice	0	0
Aloe vera gel-no copper(II)	0	0
Aloe vera gel-1% copper(II)	100	2.0
Aloe vera gel-1% tin(II)	100	2.5

It is evident from the above results that the subject invention provides compositions of aloe vera gel-ionic metal complexes for topical skin, wound and surgical treatments to protect damaged skin and facilitate natural healing processes, to enhance tissue regenerative processes in the epidermis, and to stimulate hair growth in warm blooded animals. The invention also provides economical methods for preparing and formulating the compositions for topical administration.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes f clarity of understanding, it will be bvi us that certain

changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for accelerating the healing of topical wounds in a warm-blooded animal which comprises:

administering to the wound a therapeutically effective amount of a composition which comprises an aloe gel complexed with an ionic metal.

2. The method of claim 1, wherein the ionic metal is copper(II), and therapeutically acceptable salts thereof.

3. A method for enhancing the recovery of skin of a warm-blooded animal from irritation, comprising:

administering to the skin irritation a therapeutically effective amount of a composition which comprises an aloe gel complexed with an ionic metal.

4. The method of claim 3 where the ionic metal is copper(II).

5. A method for protecting the skin from oxidative damage and aiding the recovery of skin wounds in a warm blooded animal, comprising:

administering to the skin or wound site a prophylactically or therapeutically effective amount of a composition which comprises a aloe gel complexed with an ionic metal.

6. The method of claim 5, wherein the ionic metal is copper(II), and therapeutically acceptable salts thereof.

7. A method for increasing hair follicle size and the rate of hair growth in a warm-blooded animal, comprising:

administering to the skin of said animal a composition which comprises a aloe gel complexed with an ionic metal in an amount sufficient to increase hair follicle size and the rate of hair gr wth in said animal.

8. The method of claim 7, wherein the composition is administered by injection into the skin.

9. The method of claim 7, wherein the ionic metal is copper(II) or tin(II), and therapeutically acceptable salts thereof.

10. The method of claim 9, wherein the ionic metal is tin(II).

11. A composition useful for accelerating the healing of topical wounds of a warm-blooded animal which comprises a therapeutically effective amount of a aloe gel complexed with an ionic metal.

12. The composition of claim 11, wherein the ionic metal is copper(II) or tin(II), and therapeutically acceptable salts thereof.

13. The composition of claim 11, wherein the ionic metal is copper(II).

14. The composition of claim 11, further comprising a pharmaceutically acceptable carrier.

15. The composition of claim 11, wherein the aloe gel-metal complex is present at a concentration of 10% to 50%.

16. A pharmaceutical composition for increasing hair follicle size and rate of hair growth in a warm-blooded animal, which comprises a hair growth stimulating amount of a aloe gel-ionic metal complex and a pharmaceutically acceptable carrier.

17. The pharmaceutical composition of claim 16, wherein the alo gel-ionic metal complex is present in the comp sition at a concentration of 10% to 50%.

18. The pharmaceutical composition of claim 16, wherein the ionic metal is copper(II) or tin(II), and therapeutically acceptable salts thereof.

19. The pharmaceutical composition of claim 16, which is formulated for topical administration.

20. The pharmaceutical composition of claim 16, which has been sterilized by heating or pasteurized.

INTERNATIONAL SEARCH REPORT

Inter. ...ional application No.
PCT/US95/03777

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :A61K 35/78, 33/34
US CL :424/195.1; 514/499
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/195.1; 514/499

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,465,629 (Maughan et al) 14 August 1984, column 2 lines 41-42.	11-20
X	US, A, 5,294,434 (King et al) 15 March 1994, column 6, lines 25-33	11-19
Y	US, A, 4,877,770 (Pickart) 31 October 1989, column 3 lines 32-41.	1-6
Y	US, A, 4,708,873 (Schulte) 24 November 1987, column 1, lines 55-56.	1-6
Y,P	US, A, 5,382, 431 (Pickart) 17 January 1995, column 6, line 63; column 14, claims 1-3, 7-10.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
26 MAY 1995	27 JUN 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Evelyn Huang Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

Inte. .ional application No.
PCT/US95/03777

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,164,367 (Pickart) 17 November 1992, abstract.	1-6

INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/US95/03777

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-6, 11-20

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

	I. national application No. PCT/US95/03777

BOX II. BSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-6, 11-20, drawn to method of promoting wound healing and the composition.

Group II, claim(s) 7-10, drawn to the method of promoting hair growth.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the method for promoting wound healing does not have "special technical features" that correspond to the method of promoting hair growth.